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Abstract

This study investigates the potential for physical damage caused by suspended fine sediment on gills of three macroinvertebrate species, *Hydropsyche siltalai*, *Ephemera danica* and *Ecdyonurus venosus*. Macroinvertebrate cadavers were exposed to three suspended sediment concentrations (control 3.5, low 83.7 and high 404.0 mg l^{-1}) at two velocities (low 0.19 m s^{-1} and high 0.37 m s^{-1}), for six hours in a recirculating flume. Tracheal gill surfaces were subsequently examined for evidence of physical damage using Scanning Electron Microscopy (SEM) images. Physical damage predominantly consisted of fine sediment coverage of gill surfaces, appearing as a deposited layer of sediment obscuring and potentially clogging the gill. For *E. venosus*, suspended sediment concentration influenced gill cover but velocity had no significant effect. Coverage of *H. siltalai* gill surfaces increased significantly between low and high sediment concentrations but only at the higher flow velocity. Gill coverage of *E. danica* did not differ across any sediment concentration. Results were

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consistent with reported species sensitivities to fine sediment, despite the use of cadavers. However, we found limited evidence of physical abrasion as a direct physical effect of fine sediment under the experimental conditions used.

Keywords

Aquatic insects; Suspended Sediment; Scanning Electron Microscopy; Benthic invertebrates

Introduction

The delivery of excessive fine sediment (particles <2 mm in diameter) to rivers can cause serious deleterious effects on aquatic ecosystems and is widely acknowledged to be one of the leading contributors to the degradation of rivers globally (Ritchie, 1972; Owens et al., 2005; Mathers et al., 2017a). Increasingly intensive agricultural land management, construction, mining, deforestation, and in-channel modifications, leading to bank erosion and channel incision, are some of the main anthropogenic sources contributing to increased sediment loads of rivers (Owens et al., 2005; Collins et al., 2009; Yule et al., 2010). Excess fine sediment in suspension can elevate turbidity levels (Waters, 1995), saltating particles may cause scour to periphyton and macroinvertebrates (Bilotta & Brazier, 2008) and, where hydraulic conditions permit, deposition can change river bed morphology, reducing habitat availability and dissolved oxygen exchange within interstitial pore spaces (Owens et al., 2005; Burdon et al., 2013; Wharton et al., 2017). These processes in turn can drive widespread community responses including a reduction of taxonomic and functional diversity (Larsen et al., 2011; Buendia et al., 2013; Mathers et al., 2017b).

Macroinvertebrate responses to fine sediment represent a complex mix of direct and indirect effects with these responses strongly influenced by whether the sediment is predominantly in suspension or deposited (see Kemp et al., 2011; Jones et al., 2012 for reviews). There are large bodies of evidence quantifying community responses to excessive fine sediment carried in suspension (Gray & Ward, 1982; Couceiro et al., 2010; Béjar et al., 2017) and deposited on and within the river bed (Larsen et al., 2011; Wagenhoff et al., 2012; Elbrecht et al., 2016; Beermann et al., 2018). There is also evidence of behavioural responses to excessive fine sediment, such as drift and vertical avoidance, although the mechanisms responsible for these changes remain uncertain (Doeg & Milledge, 1991; Larsen & Ormerod, 2010). Research has quantified the effects of suspended sediment on feeding efficiency (Kefford et al., 2010), egg survival (Everall et al., 2018), and the effect of burial by sediment deposition (Wood et al., 2005; Conroy et al., 2018). However, thus far research which considers the direct physical effects of fine sediment in suspension at the organism level is limited. Based on this

58 evidence, there are likely to be two main processes through which suspended sediment affects
59 macroinvertebrates physically: (i) coverage of fine sediment on tissues and external structures,
60 potentially leading to clogging effects; and (ii) abrasion - physical damage in the form of scrapes or
61 scratches from the angularity of fine sediment particles in suspension or saltation.

62

63 Clogging effects from fine sediment were first defined by Lemly (1982) as the accumulation of
64 particles on body surfaces and respiratory structures. These effects have been reported in fish,
65 affecting gaseous exchange through the gill epithelium and disrupting respiration (Cordone & Kelley,
66 1961; Bond & Downes, 2003) and osmoregulation (Bruton, 1985; Waters, 1995; Bergstedt &
67 Bergersen, 1997). Similarly, for macroinvertebrates, fine sediment can also build-up on external organ
68 surfaces and disrupt the normal functioning of gills and filter-feeding apparatus (Strand & Merritt,
69 1997; Allan, 2004). The rationale linking the effects of fine sediment to clogging predominantly
70 concerns filter feeders that may spend extra time expelling unwanted inorganic particles (e.g.
71 Molluscs - MacIsaac & Rocha, 1995) and cleaning filter feeding structures (e.g. Cladocera - Arruda et
72 al., 1983; Hart, 1992). In extreme instances, filter feeders may become excluded from habitats
73 receiving high inputs of fine sediment (e.g. Armitage & Blackburn, 2001).

74

75 Abrasion caused by fine sediment has been referred to in the literature multiple times, yet the primary
76 scientific evidence appears limited. First reported to affect macrophytes subject to excessive
77 suspended sediment concentrations (SSC) downstream of mining activities (Lewis, 1973a, 1973b),
78 abrasion has been cited as affecting benthic assemblages and algae (Bond & Downes, 2003;
79 Francoeur & Biggs, 2006) and causing damage to soft tissues and gills in fish (Herbert & Merkins,
80 1961; Kemp et al., 2011) and fine and fleshy body parts in macroinvertebrates (Jones et al., 2012;
81 Wharton et al., 2017). The abrasion hypothesis has been linked to behavioural responses such as
82 retraction of feeding apparatus or changes to feeding mechanisms, avoidance behaviour, and passive
83 or active drift (Bilotta & Brazier, 2008).

84

Abrasion and clogging as causes of macroinvertebrate responses to fine sediment remains largely hypothetical and based on correlative evidence due to the difficulties of quantifying the physical effects in real time by direct observation (Jones et al., 2012). This study aims to build on more specific exposure experiments, such as Rosewarne et al. (2014) who exposed white-clawed crayfish [*Austropotamobius pallipes* (Lereboullet, 1858)] and signal crayfish [*Pacifastacus leniusculus* (Dana, 1852)] to varying concentrations of fine sediment. The results showed increased gill clogging at higher concentrations of fine sediment. In the current laboratory flume experiment, we aimed to investigate the physical effects of fine sediment carried in suspension on cadaver macroinvertebrate gills of three species with varying gill morphologies; branched gills of *Hydropsyche siltalai* Doehler, 1963 (Trichoptera: Hydropsychidae), feathery gills of *Ephemera danica* Müller, 1764 (Ephemeroptera: Ephemeridae) and plate-like gills of *Ecdyonurus venosus* (Fabricius, 1775) (Ephemeroptera: Heptageniidae). Our objectives were to: (1) characterise and quantify any potential damage to macroinvertebrate gills through sediment coverage or abrasion of gill surfaces; (2) investigate the effect of increasing SSC and flow velocity on the extent of physical cover and damage observed; and (3) assess whether physical damage varies between gill type and structure (species). We hypothesised that physical effects would be influenced by both SSC and flow velocity. Specifically, we hypothesised that coverage of fine sediment on gill surfaces would increase at higher SSC and that damage associated with abrasion would be greater at higher flow velocities as a result of the higher impact speed of sediment particles.

Observing the effects of fine sediment on live macroinvertebrates presents unique challenges due to known behavioural responses to disturbance. During exposure to fine sediment in the experimental procedure, live individuals may attempt drift or seek refuge on the bed or margins of the flume (Bilotta & Brazier, 2008). Alternatively, the use of microcosms to restrict movement within a defined area would have resulted in disruption of hydraulic characteristics. In both instances, live individuals would be free to move, change body position and find the most preferable refuge location within the flume in order to avoid the potential physical effects of fine sediment. As a direct result of the potential confounding effects due to the movement and avoidance behaviour (including drift out of

113 the flume) of live invertebrates, we decided to use immobile cadavers to provide control over the
114 nature of exposure to elevated suspended sediment (location in the main flow, body position and
115 alignment in relation to flow direction). This control ensured that all of the invertebrates (and hence
116 gills) were exposed to the main flow and sediment within the flume in a similar manner throughout
117 the experimental period, providing a benchmark from which we could determine any physical effect
118 of fine sediment on gill surfaces. Therefore, through the results of this study, we hope to build on the
119 understanding of the mechanisms behind macroinvertebrate responses to fine sediment, a topic which
120 requires further research (Wilkes et al., 2017), as well as provide additional insight on potential
121 advances in methodology and techniques to further study the effects of fine sediments on
122 macroinvertebrates.
123

124 **Materials and methods**

125 Macroinvertebrate specimens were collected from a second order lowland stream (Woodbrook,
126 Leicestershire, UK, 52°75' N, -1°21'W) in May 2017. Substrates were gently disturbed and drifting
127 insects captured with a pond net (mesh size 1 mm) thereby minimising damage to gills. Specimens
128 were immediately transferred to 70% industrial methylated spirit (IMS) to preserve and transferred to
129 distilled water a few hours prior to experiments to ensure a buoyancy identical to that in the
130 experimental flume. All cadavers were examined with the aid of a dissecting microscope prior to use
131 in experiments to ensure that gills were intact and that there was no damage or abnormalities, and
132 only those that had no signs of damage were used in experiments. During all stages of the
133 experimental procedure, cadavers were handled using soft watch-spring non-serrated forceps and the
134 abdomen and thorax were avoided when handling to minimise any damage to gills.
135

136 Cadavers were exposed to three SSC levels (mean ± SD): 3.5 ± 0.96 mg l^{-1} (control), 83.7 ± 7.74 mg l^{-1}
137 (low) and 404.0 ± 77.25 mg l^{-1} (high); and two flow velocities (0.19 m s^{-1} and 0.37 m s^{-1}) in a full
138 factorial design. Due to the difficulties in measuring SSC continuously, we used turbidity as a
139 surrogate. The three SSC levels corresponded to turbidity values of <2.5 NTU (control), 100 NTU and

400 NTU. The SSC levels were selected to represent the range of natural conditions typically encountered in lowland UK rivers (Bilotta et al., 2012; Grove et al., 2015), and flow velocities were representative of the selected taxa preferences (Tachet et al., 2010).

Experimental procedure

Experiments were conducted in a large recirculating flume system (flume dimensions 10 m long x 0.3 m wide x 0.5 m deep) thereby minimising potential edge effects present in smaller systems. The flume was filled with tap water and water temperature was allowed to fluctuate under ambient air conditions (21.47 ± 0.60 °C). Macroinvertebrate cadavers were pinned to cork tiles (300 mm x 300 mm) fitted flush to the base of the flume. Each cadaver was positioned in the same dorso-ventral body posture (facing the flow) such that exposure to the suspended sediment was consistent amongst all individuals (not possible with live individuals). Each experimental trial exposed six macroinvertebrate cadavers of each species for six hours. Based on field-based research in local streams, SSC peaks approximate those recorded in the field (Mathers, 2017). The experimental area (i.e. cork tiles) was located 6 m from the header tank. Textured sand boards were placed around the experimental area to create natural surface roughness and turbulence and the cadavers were located in the central third of the experimental area to reduce any effects of the flume walls. Each cadaver was positioned ~ 3.5 times their average body length away from each other in two rows. This configuration mitigated any hydraulic effects from the flume walls and ensured fully developed flow over the experimental area (Lacey et al., 2012). Given that the configuration was based on empirical scalings describing the dimensions of turbulent structures around bluff bodies (Wilkes et al., 2013) it also mitigated for any hydraulic effects between cadavers in the same experimental run. Given the configuration of the flume and the spacing between cadavers and solid boundaries, each cadaver can be considered statistically independent within the same trial. Following the experimental run cadavers were carefully removed and placed in individual vials of 70% IMS.

For the SSC treatments, a fluvial sediment aggregate mixture (average organic component of 7.70 ± 1.16%, particle size D_{10} 10.41 µm, D_{50} 221.40 µm, D_{90} 505.43 µm; see below for particle size analysis method) was gradually wet sieved to 500 µm directly into the holding tank until the required turbidity was achieved. Turbidity was monitored at 1 s intervals using a Eureka 2 Manta sonde fitted with a self-wiping function (International Organisation for Standardisation 7027; 0-3000 NTU, quoted error ± 1%) to ensure turbidity remained consistent throughout the experimental period of six hours. If levels dropped below 95% of the target value, additional fines were added as required. The turbidity would initially peak after sediment addition and as such time was allowed for mixing between each new addition. Turbidity levels were stabilised at the required level before the start of each experimental trial. Despite excluding larger fractions of fine sediment (0.5 µm – 2 mm), this provided an opportunity for creating conditions analogous to natural riverine conditions since it is this finer fraction which dominates suspended sediment load (Church et al., 1987; Chang, 1998). The depth of water within the flume was maintained at 100 mm (± 10 mm) above the bed and velocity measured at 0.6 depth at 12 locations over the experimental area (Valeport electromagnetic current meter) during each trial.

Turbidity measurements are sensitive to the physical characteristics of the sediment (Bilotta & Brazier, 2008) and therefore SSC was measured for validation. During each experimental trial, three 1 L samples of water were collected from the flume immediately downstream of the experimental area. This procedure was repeated three times for each trial (just once for the control). Samples were filtered using Whatman 0.7µm glass microfiber filters and analysed for dry weight mass including percent organic matter through loss-on-ignition (Dean, 1974). Mean turbidity and SSC for each experimental trial are provided in Table S1. Laser particle size analysis (Malvern Mastersizer 2000) was used to obtain the particle size distribution of the sieved sediment aggregate mix (<500 µm). The sediment was prepared by first removing organic matter by adding 5 ml of 30 % hydrogen peroxide to ~ 0.5 g sediment in a test tube. After 24 hours, the samples were heated to 70 °C until no gas bubbles were released from the mixture. Five ml of 3% sodium hexametaphosphate (Calgon) were added to

193 disperse the particles (Gray et al., 2010). Each sample was subjected to two minutes of ultrasonic
194 dispersion immediately prior to analysis and measured for a total of 60 s at 8-12% obscuration (Blott
195 et al., 2004). A particle size distribution curve is provided in Figure S1.

196

197 Microscopy procedure

198 For an overview of sediment coverage on macroinvertebrate gill surfaces, individual gills from
199 cadavers within each treatment were mounted on microscope slides using Hoyer's solution. Images of
200 the gills from each slide were examined using a stage microscope. Images were taken using a Nikon
201 eclipse 80i (for examples see Figure S2). The fine sediment accumulation on each individual gill was
202 visually assessed qualitatively by examining individuals used in experiments using a dissecting
203 microscope and found to be consistent across all gills of each individual, within each treatment. As a
204 result, only two gills from a single individual of each species from each treatment were used for
205 detailed examination.

206

207 For detailed gill surface profile images, Scanning Electron Microscopy (SEM) was used. Individual
208 gills were carefully removed from cadavers from each experimental trial using soft watch-spring
209 forceps. The gills were prepared for SEM by freeze-drying overnight (CHRIST BETA 1-8 LDplus
210 Freeze Drier). A pilot experiment, conducted in order to determine the correct preparation method
211 prior to SEM, yielded images of *Ecdyonurus venosus* directly from the river after preservation in IMS
212 (i.e. not exposed to any treatment). These 'control' images indicated little sediment on the gill
213 surfaces and confirmed that any sediment accumulated on the gill surface of the test individuals was
214 the result of direct physical effects from exposure (see Figure S3).

215

216 For *Ecdyonurus venosus* gills five and six were used, whereas gills five and eight were used for
217 *Hydropsyche siltalai* and gills four and six for *Ephemera. danica*. The selection of these particular

gills was made because they were intact across all individuals within each species. An additional step was required to prepare gills for the investigation of physical damage by abrasion, in order to remove the fine sediment adhered to the surface of the gills. One individual of each species from each treatment was placed in an ultrasonic bath (Fisherbrand* FB11004) for two 30 s periods (at 100% - standard setting), sufficient to remove adhered fine sediment but low enough to not cause any physical damage in the process. Gills were sputter-coated in Gold-Palladium for 90 seconds prior to analysis.

In order to ensure consistency for subsequent image analysis, images were captured on areas of the gill surface where the following criteria were satisfied: the gill surface filled the whole frame; the aspect of the surface was normal to the optical axis; and the area was representative of the coverage on the gill surface and away from the gill margin. Three images were taken of each gill, at different locations on the surface, at 5,000 X magnification for *Ecdyonurus venosus* and *Ephemera danica* and the higher magnification of 25,000 X for the smaller gills of *Hydropsyche siltalai*. These magnifications allowed us to meet the above criteria. However, some SEM images did not meet these criteria and were discarded. For images used to quantify sediment coverage of gill surfaces, this left 31 images for *E. danica*, 33 for *E. venosus* and 36 for *H. siltalai*. All images were retained for assessing physical damage by abrasion (36 for each species).

In order to determine and confirm the appearance of sediment particles, fine sediment samples collected from the macroinvertebrate sample site in the field (during macroinvertebrate collection) and from the experimental sediment aggregate mix were oven-dried overnight, sieved to 500 μm and processed for SEM examination using the method outlined above.

Image analysis

The resulting images of gills were used to characterise the extent of sediment-surface coverage and abrasion. To reduce subjectivity from visual assessments, a non-automated digital image analysis technique developed and described in Turley et al. (2017) was used. The method was developed in order to reduce variability from purely visual estimate-based methods of sediment-surface cover on river beds. In the original publication from which the method originates, the inter-operator variability of digital analysis was shown to be 5% compared to visual estimates which can have up to 40% inter-operator variability (Duerdoth et al., 2015). Areas of sediment coverage were highlighted by the same operator throughout the process using the foreground colour (#FA0200) in Adobe Photoshop. Each image was then exported and uploaded to *PixelCount* (Turley et al., 2017), a software application that calculates the percentage of each image highlighted in a selected colour, thereby providing the percentage of sediment cover on each image. Bacteria on the gill surfaces, identified as rod-shaped particles (Lemly, 1982), were not highlighted. Examples illustrating the varying percentage of sediment cover are shown in Figure 1. Abrasion was assessed using a visual assessment of the images in which all areas of abnormal gill surface textures and marks were recorded.

Statistical analysis

Percentage data (percentage of sediment coverage) was arcsine square root transformed prior to analysis. A three-way unbalanced ANOVA (Akritas et al., 1997) was used to test for significant effects of species, SSC, flow velocity and all interactions in relation to the surface area of the gill image covered by fine sediment. The resulting nested models were compared separately for each species using an F-test. Pairwise post-hoc Tukey's HSD tests were carried out using the *glht* function from the *multcomp* package in R (Hothorn et al., 2008). Given the relatively small sample size, and the fact that fine sediment accumulation was consistent across all gills of each individual within each treatment, gill number was not included as a random effect. All statistical analyses were carried out using R version 3.4.4 (R Development Core Team, 2019).

Results

The physical effects of fine sediment on the individual gill tissues predominantly consisted of fine sediment-cover on the gill surface (Figure 2). Chloride cells (structures used for osmoregulation) were observed on the SEM images of both *Ephemera danica* and *Ecdyonourus venosus* (white circles, Figure 2). For *E. danica* these were covered by sediment to some degree under all experimental conditions, but for *E. venosus* these remained clear for the control conditions. The texture of sediment particles covering gills was consistent with that of the fine sediment particles from the experimental sediment aggregate mix and those collected from the macroinvertebrate sample sites (Figure 3). The extent to which the gill was covered varied by sediment concentration and the morphology of the gills of the different species used (Figure 4). A three-way ANOVA demonstrated sediment cover on the gill surface did significantly vary as a function of species ($F_{2,82}$=29.50, $p<0.001$), sediment ($F_{2,82}$=21.41, $p<0.001$), and species:sediment ($F_{4,82}$=8.67, $p<0.001$), species:velocity ($F_{2,82}$=5.67, $p<0.001$) and three-way ($F_{4,82}$=5.62, $p<0.001$) interactions (Table S2). The sediment:velocity interaction was not significant ($F_{2,82}$=0.96, $p=0.39$) across all species. Neither was this interaction significant for *E. venosus* ($F_{2,27}$=1.53, $p=0.23$) or *E. danica* ($F_{2,25}$=1.37, $p=0.27$). However, the model including the sediment:velocity interaction for *Hydropsyche siltalai* was significant ($F_{2,30}$=9.76, $p<0.001$) (Table S3). Post-hoc tests indicated significantly more fine sediment coverage for *E. venosus* as SSC levels increased but no significant effect of velocity (Table 1). In contrast, there were no significant effects of either SSC or flow velocity on gill cover in *E. danica*. The only significant result for *H. siltalai* was a significant increase in fine sediment coverage between low (83.7 mg l^{-1}) and high SSC (404.0 mg l^{-1}) only when velocity was low (0.19 m s^{-1}) (Figure 4; Table 1). Physical damage in the form of abrasion was evident in two images, one for *E. venosus* and one for *E. danica*. In these instances, marks on the surface of gills appeared to be inconsistent with normal gill texture appearance, potentially indicating abrasion from sediment particles (Figure 5). No abrasion was observed on gills of *H. siltalai*.

Discussion

296 This study aimed to investigate the physical effects of suspended fine sediment at differing flow
297 velocities on the gills of cadavers from three common species of lotic macroinvertebrates. We
298 hypothesised that increasing SSC and flow velocity would affect the extent of physical damage in the
299 form of sediment coverage of macroinvertebrate gill surfaces. We found evidence that partially
300 supports this, with gill coverage in *Ecdyonurus venosus* increasing significantly with SSC. Gill
301 coverage in *Hydropsyche siltalai* was only significantly different between low and high SSC
302 treatments when flow velocity was low (this was not the case when velocity was high). Velocity did
303 not affect gill coverage for any other species. There was no effect of any sediment concentration on
304 gill coverage in *Ephemera danica.* We also hypothesised that increasing velocity would lead to
305 increased abrasive damage to gill surfaces. Abrasion was only observed in two instances, hence we
306 found little support for this second hypothesis.

307

308 Fine sediment coverage in *Ecydonurus venosus* appeared to increase linearly with SSC. The gills of
309 *Ephemera danica* were consistently covered with fine sediment across all three SSC treatments. The
310 fine sediment coverage of *Hydropsyche siltalai* gills appeared linear when flow velocity was slower.
311 However, this relationship was not observed at the higher flow velocity. Species identity was
312 significant in predicting sediment cover, and gills of *H. siltalai* had lower sediment coverage across
313 all the treatments compared to the other species.

314

315 In the closed tracheal system of aquatic insects, respiration occurs through tracheal gills which vary in
316 structure by macroinvertebrate order and family level. This variation can partially help explain the
317 results recorded. All six pairs of *Ephemera danica* gills are bilamellated, feather-like and oscillate in
318 synchronous pairs creating a water current over the dorsal side of the body between the two rows of
319 gills (Eastham, 1939). During the experimental procedure, gills were positioned upwards
320 perpendicular to the body in the water column, directly exposed to fine sediment in suspension and
321 saltating over the bottom of the flume. The small feathering branches on each tracheate gill effectively

322 became nets for fine sediment which was evident with high sediment coverage recorded even for the
323 control trials. *Ecdyonurus venosus* gills are held to the side of the abdomen and project downwards.
324 Pairs 1-6 consist of a lamelliform gill plate and a proximal gill tuft underneath, whilst gill 7 comprises
325 a single gill plate (Eastham, 1937). The gill plate was analysed for the study as this portion of the
326 tracheal gill is exposed to the flow and fine sediment in suspension. The gills stayed relatively
327 stationary during the experimental procedure and exhibited increasing sediment coverage with SSC.
328 *Hydropsyche siltalai* gills consist of a few, pale, branched gill tufts held under the abdomen. This
329 species exhibited lower gill sediment coverage than the two Ephemeroptera species. Hydropsychidae
330 gills are located under the abdomen which potentially provides protection from physical damage by
331 suspended sediment.

332

333 Ecological interpretations

334 It should be noted that for the practicalities of this study, we used cadavers to determine the physical
335 effects of suspended sediment on macroinvertebrates (gill coverage and abrasion). Where historically
336 the deposition of particles on the surface of gills has been defined as 'clogging', we have defined
337 potential damage as fine sediment 'coverage' of gills. This is because it cannot be confirmed whether
338 sediment coverage on gill surfaces directly equates to impaired functioning of key structures involved
339 in respiration and osmoregulation through the use of cadavers. Additionally, the individuals were not
340 able to exhibit avoidance behaviours such as active drift (Doeg & Milledge, 1991; Larsen & Ormerod,
341 2010) or able to clean sediment covered structures (Eastham, 1939). However, the results from this
342 study are intuitive based on the traits and preferences of the test species which we explain below, and
343 do provide the opportunity to directly study the mechanisms of potential gill impairment which would
344 not be possible through the use of live individuals

345

346 *Ephemera danica* gills were covered with fine sediment consistently regardless of the experimental
347 trial. This species displays habitat preference for sand, silt and clay substrates within which the

348 organism burrows (Elliott & Humpesch, 2010). All *Ephemera* spp. display trait characteristics
349 associated with life in fine sediment deposits, with modified mouthparts, processes on the head, and
350 broadened prothoracic legs which allow them to excavate and burrow into the substrate (Eriksen,
351 1963; Elliott & Humpesch, 2010). The presence of numerous hairs on the gills prevent fine sediment
352 particles from completely smothering them (Hynes, 1970) and the setae brushes on the rear legs are
353 used to clear body parts of accumulated debris (Eastham, 1939). *E. danica* is therefore considered
354 relatively tolerant of high fine sediment concentrations (Bennett, 2007; Extence et al., 2013).

355

356 *Ecdyonurus venosus* is widely described as a clinger and lives on rocks and other hard substrates. It is
357 adapted to live in close association with high flow velocities and shear stresses (Lancaster & Belyea,
358 2006), and avoids dislodgment from substrates by being dorsoventrally flattened and possessing large
359 curved tarsal claws to cling on to hard substrates (Wichard et al., 2002; Elliott & Humpesch, 2010).
360 The role of its lamelliform gill is to generate a current and draw oxygen in, whereas the filamentous
361 sections are for respiration (Eastham, 1937). For *E. venosus*, the lamelliform gill provides some
362 protection from fine sediment to the filamentous gills underneath. Consistent with these
363 characteristics and the results of previous biomonitoring studies (e.g. Murphy et al., 2015; Turley et
364 al., 2016), our findings supported the classification of *E. venosus* gill surfaces as sensitive to fine
365 sediment.

366

367 *Hydropsyche siltalai* typically constructs feeding nets either side of a tubular retreat (Edington &
368 Hildrew, 1995). These structures are either exposed (at right angles to the local flow) or in crevices
369 beneath and underneath stones where gravel and plant material can be used as support. Particles
370 caught in the net are collected using the mandibles and prothoracic legs, whilst inedible particles are
371 ejected (Edington & Hildrew, 1995). In environments characterised by high availability of fine
372 sediment, these nets become clogged causing the organism to spend increasing amounts of time
373 cleaning the nets or in extreme instances abandoning the nets (Strand & Merritt, 1997). Although it is

374 regarded as moderately sensitive to fine sediment (Murphy et al., 2015; Turley et al., 2016), *H. siltalai*
375 had relatively low coverage of sediment of gills across all trials, suggesting that sensitivity in this
376 species is probably primarily associated with the filter feeding mechanism and/or cleaning of nets.

377

378 Potential biological implications

379 Respiration and osmoregulation are intimately associated processes in aquatic organisms and essential
380 to inhabiting aquatic environments (Wichard et al., 2002). During respiration, through the diffusion of
381 oxygen in to the insect, water also penetrates by osmosis. Excess water is excreted by the body and
382 the re-uptake of ions is carried out by specialised chloride cells which are usually located on the gills.
383 Chloride cells which become clogged with fine sediment will ultimately affect osmoregulation
384 (Bruton, 1985; Waters, 1995; Bergstedt & Bergersen, 1997). However, chloride cells can vary in
385 number depending on water salinity (Wichard et al., 1973), and it could therefore be possible that at
386 continually high SSC levels when gills are likely to be heavily covered by fine sediment (and function
387 inhibited), chloride cell densities can increase. Trichopterans do not possess chloride cells and instead
388 the uptake of ions is carried out by other forms, predominantly through chloride epithelia (Wichard et
389 al., 1973, 2002). Possessing a range of methods of ion re-uptake may indicate osmoregulation is less
390 affected by fine sediment deposition and coverage of gills and other body parts for trichopterans.
391 Whilst studying the effect of aluminium on gills of *Ephemera danica*, Herrmann and Andersson
392 (1986) noted mucus formation on the gills during exposure. The result of this mucus formation was to
393 impair osmoregulation and lower respiration efficiency, causing the mayfly to increase respiration to
394 compensate. It is unknown whether insect larvae can secrete mucus for gill protection as a result of
395 abrading sediment, as is the case for fish gills (McCubbin et al., 1990). However, in high sediment
396 conditions, the mucus secretions resulted in increased susceptibility to coverage of sediment on the
397 gill surface and ultimately suffocation of the fish.

398

399 Limitations and future research

400 This study provides evidence of the effect of varying levels of fine sediment suspension on
401 macroinvertebrate gills of specific taxa using a novel methodological approach, through SEM and
402 image analysis, that can be applied in freshwater research to produce quantifiable results. It is
403 recognised that there is some subjectivity in the imaging process, although the systematic digital
404 image analysis process employed minimises such subjectivity in the assessment of fine sediment
405 coverage. We therefore suggest that this SEM application provides a robust estimate of fine sediment
406 coverage of gill surfaces. We recommend that the results should be treated with caution when applied
407 to natural conditions due to the experimental use of cadavers. Closed chamber methods, using live
408 insect larvae, could be used to confirm whether fine sediment coverage on insect gills has a negative
409 effect on respiration (Rostgaard & Jacobsen, 2005). Abrasion appeared to be less important when
410 considering the effects of physical damage from fine sediment, although further research is required to
411 assess its prevalence with varying levels of angularity, particle size and water velocities. This research
412 will help us understand how aquatic macroinvertebrates respond to excess fine sediment and the traits
413 we need to consider to improve fine sediment-specific biomonitoring tools (Wilkes et al. 2017).

414

415 **Conclusion**

416 Studies assessing the direct and physical impacts of fine sediment for macroinvertebrates at the
417 organism level have been relatively limited to date. This experiment has, for the first time,
418 demonstrated the potential physical effects of fine sediment on macroinvertebrate gill surfaces,
419 through fine sediment coverage and abrasion, in cadavers of three species of lotic macroinvertebrates.
420 In contrast to the widely cited effects of abrasion in the literature, we found evidence that gill
421 coverage was the primary effect, with abrasion only recorded in two instances. However, increasing
422 SSC was associated with increased gill coverage for only one species (*Ecdyonurus venosus*). Flow
423 velocity and species' traits and ecology interacted to produce a variable response to fine sediment.
424 Although these results must be interpreted with caution given the use of cadavers, these differences
425 can be explained by variations in gill structure, and in relation to known species' habitat preferences
426 and traits.

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625

626 **Tables**

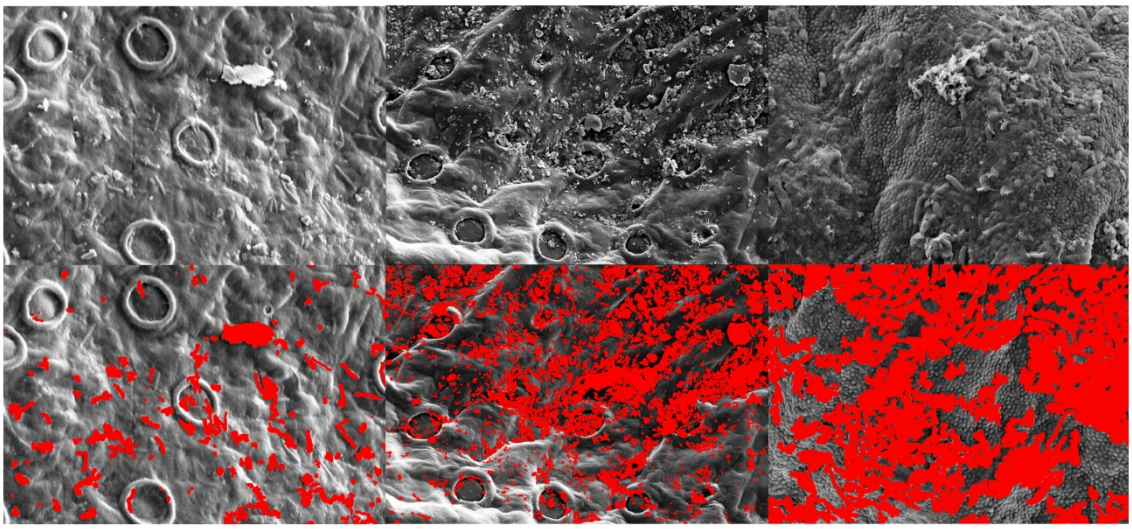
627 Table 1. Summary results from the post-hoc general linear hypothesis tests. *Denotes a significant
628 term ($p < 0.05$).

Hypothesis	**Estimate**	**SE**	***t***	***p***
Ecdyonurus venosus				
Sediment: 404.0 mg l^{-1} – Control = 0	0.53	0.05	9.98	<1e-03*
Sediment: 83.7 mg l^{-1} – Control = 0	0.31	0.05	5.66	<1e-03*
Sediment: 83.7 mg l^{-1} – 404.0 mg l^{-1} = 0	-0.22	0.05	-4.29	<1e-03*
Velocity: 0.19 m s^{-1} – 0.37 m s^{-1} = 0	-0.09	0.04	-2.19	0.12
Ephemera danica				
Sediment: 404.0 mg l^{-1} – Control = 0	0.02	0.09	0.22	0.99
Sediment: 83.7 mg l^{-1} – Control = 0	-0.09	0.09	-0.98	0.72
Sediment: 83.7 mg l^{-1} – 404.0 mg l^{-1} = 0	-0.11	0.08	-1.33	0.50
Velocity: 0.19 m s^{-1} – 0.37 m s^{-1} = 0	0.15	0.07	2.23	0.11
Hhydropsyche siltalai				
0.19 m s^{-1}: 404.0 mg l^{-1} – Control = 0	0.22	0.09	2.49	0.09
0.19 m s^{-1}: 83.7 mg l^{-1} – Control = 0	-0.22	0.09	-2.50	0.09
0.19 m s^{-1}: 83.7 mg l^{-1} – 404.0 mg l^{-1} = 0	-0.43	0.09	-4.99	1.33-04*
0.37 m s^{-1}: 404.0 mg l^{-1} – Control = 0	-0.03	0.09	-0.34	1.0
0.37 m s^{-1}: 83.7 mg l^{-1} – Control = 0	0.08	0.09	0.90	0.87
0.37 m s^{-1}: 83.7 mg l^{-1} – 404.0 mg l^{-1} = 0	0.11	0.09	1.25	0.67

629

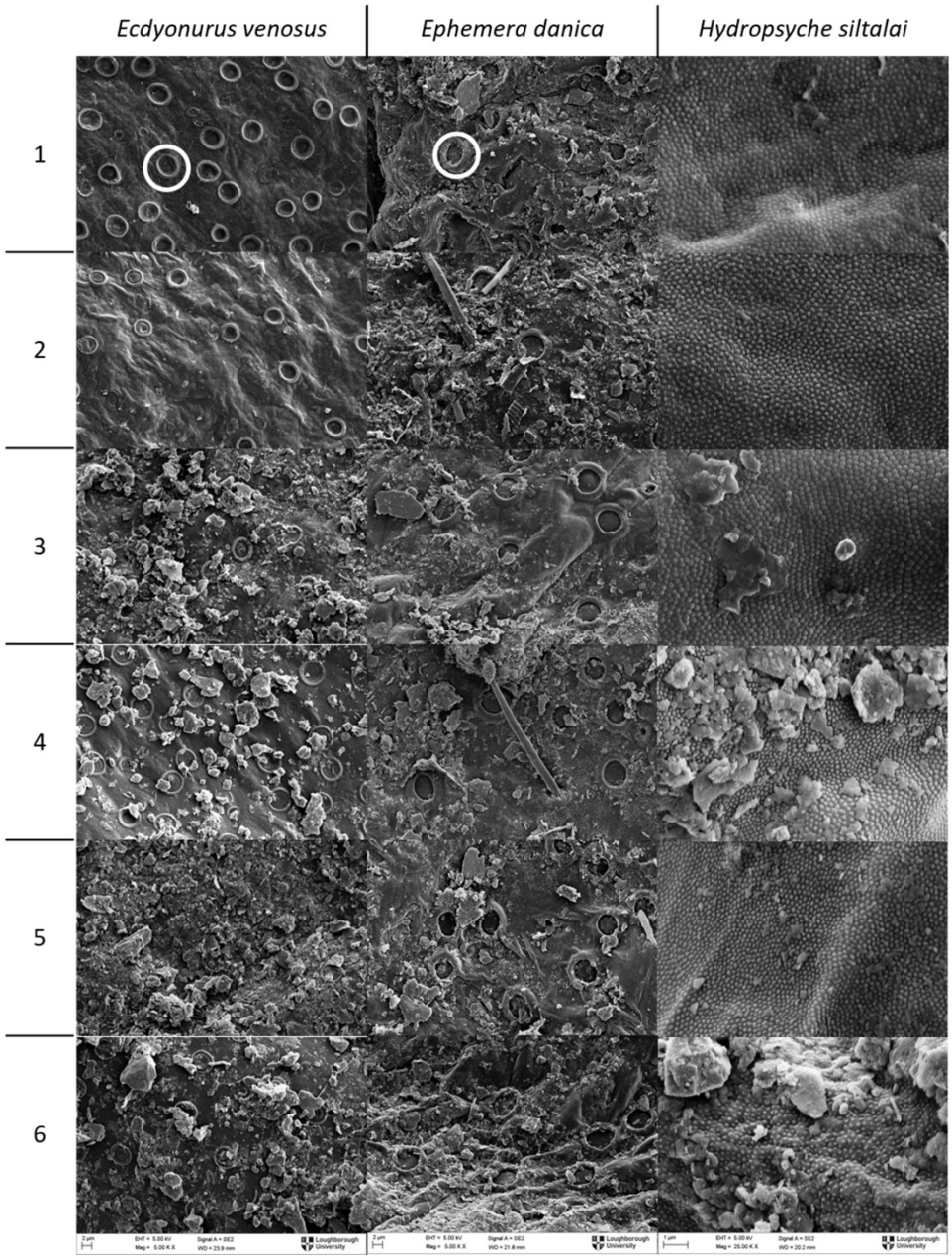
630

631 **Figures and figure captions**



632

633 Figure 1. Images showing the digital image analysis process with examples from each test species;
634 *Ecdyonurus venosus*, *Ephemera danica* and *Hydropscyhe siltalai*. The top row shows the original
635 SEM images and the bottom row the same images after digital image analysis (with sediment particles
636 highlighted in red). The percentages below the images equate to the total area per frame covered with
637 fine sediment (which is calculated from the percentage of image highlighted in red).



638

639 Figure 2. Scanning Electron Microscope images for *Ecdyonurus venosus* (images at 5,000
640 magnification), *Ephemera danica* (images at 5000 X magnification) and *Hydropscyhe siltalai* (images
641 at 25,000 X magnification) after exposure to two controls and four treatments of varying SSC and
642 flow velocity. Control (1) = 3.5 mg l^{-1} SSC at 0.19 m s^{-1}, control (2) = 3.5 mg l^{-1} SSC at 0.37 m s^{-1},
643 treatment (3) = 83.7 mg l^{-1} SSC at 0.19 m s^{-1}, treatment (4) = 83.7 mg l^{-1} SSC at 0.37 m s^{-1}, treatment
644 (5) = 404.0 mg l^{-1} SSC at 0.19 m s^{-1} and treatment (6) = 404.0 mg l^{-1} SSC at 0.37 m s^{-1}. An example of
645 a chloride cell is circled in white for the two Ephemeroptera species, *E. venosus* and *E. danica,* in the
646 images from treatment one.

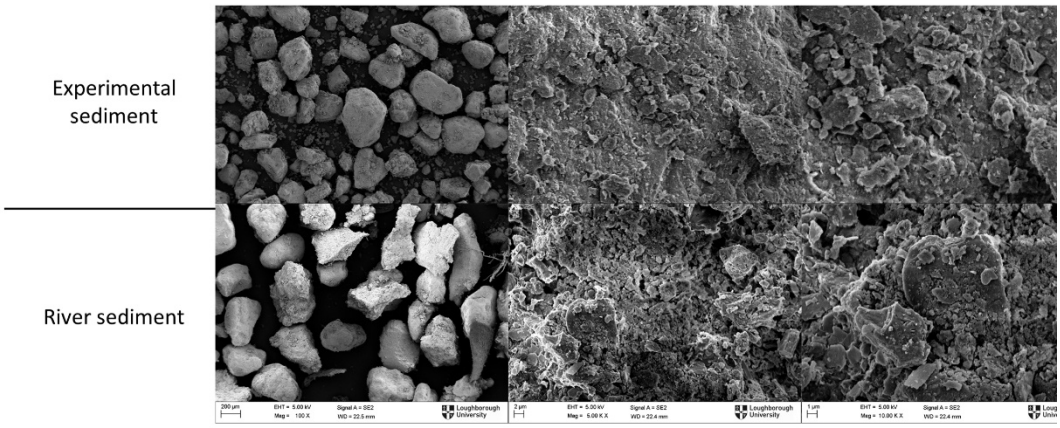


Figure 3. Scanning Electron Microscope Images of the sediment aggregate mix (used in the experimental treatments – top) and natural riverine sediment (collected from the macroinvertebrate collection sites – bottom) at increasing magnifications (left to right); 100 X, 5,000 X and 10,000 X.

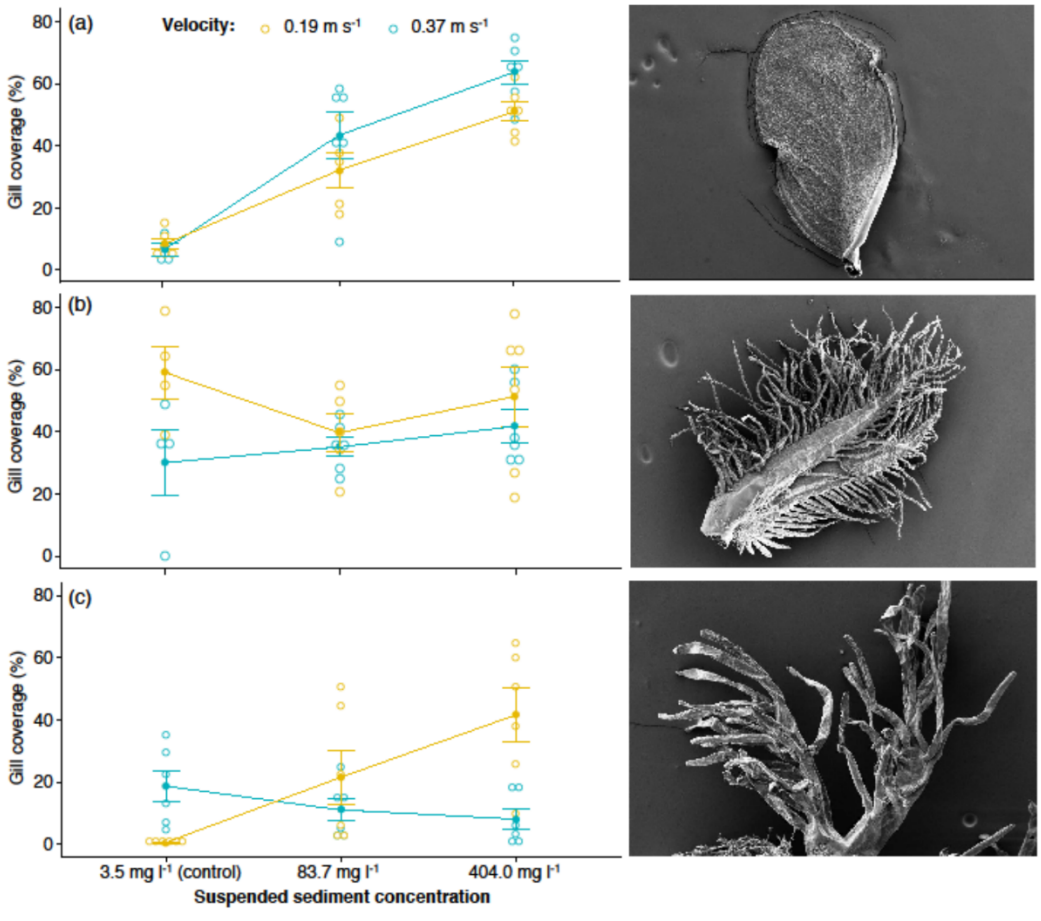
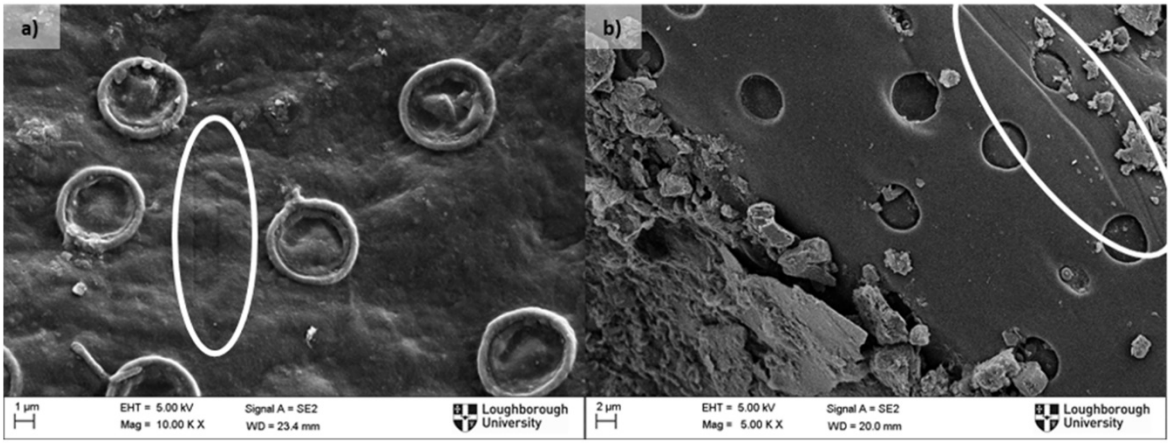


Figure 4. Percentage gill coverage between experimental trials and SEM images of the entire gill structures for a) *Ecdyonurus venosus*, b) *Ephemera danica* and c) *Hydropscyhe siltalai*. Filled circles show the mean values for each treatment.



655

656 Figure 5. Possible evidence of abrasion seen as striations (within white circled areas) on a) *Ephemera*
657 *danica* (83.7 mg l^{-1} SSC and 0.19 m s^{-1} without ultrasonic treatment) and b) *Ecdyonurus venosus* (3.5
658 mg l^{-1} SSC and 0.37 m s^{-1} with ultrasonic treatment).

659

Mckenzie et al. Supplementary material.

Table S1. Target turbidity, mean turbidity (from 1 s resolution sonde data), mean suspended sediment concentrations and mean velocity (± 1 standard deviation) for each experimental trial.

Trial	Target turbidity (NTU)	Mean turbidity (NTU)	Mean suspended sediment concentration (mg l^{-1})	Mean velocity (m s^{-1})
1	< 2.5	1.29 (0.12)	3.82 (1.32)	0.19 (0.003)
2	< 2.5	2.76 (0.41)	3.19 (3.19)	0.41 (0.01)
3	100	101.27 (5.61)	81.02 (7.94)	0.19 (0.004)
4	100	101.94 (4.38)	86.31 (6.55)	0.34 (0.01)
5	400	401 (11.68)	368.52 (42.05)	0.19 (0.01)
6	400	399.49 (8.90)	439.97 (88.39)	0.35 (0.01)

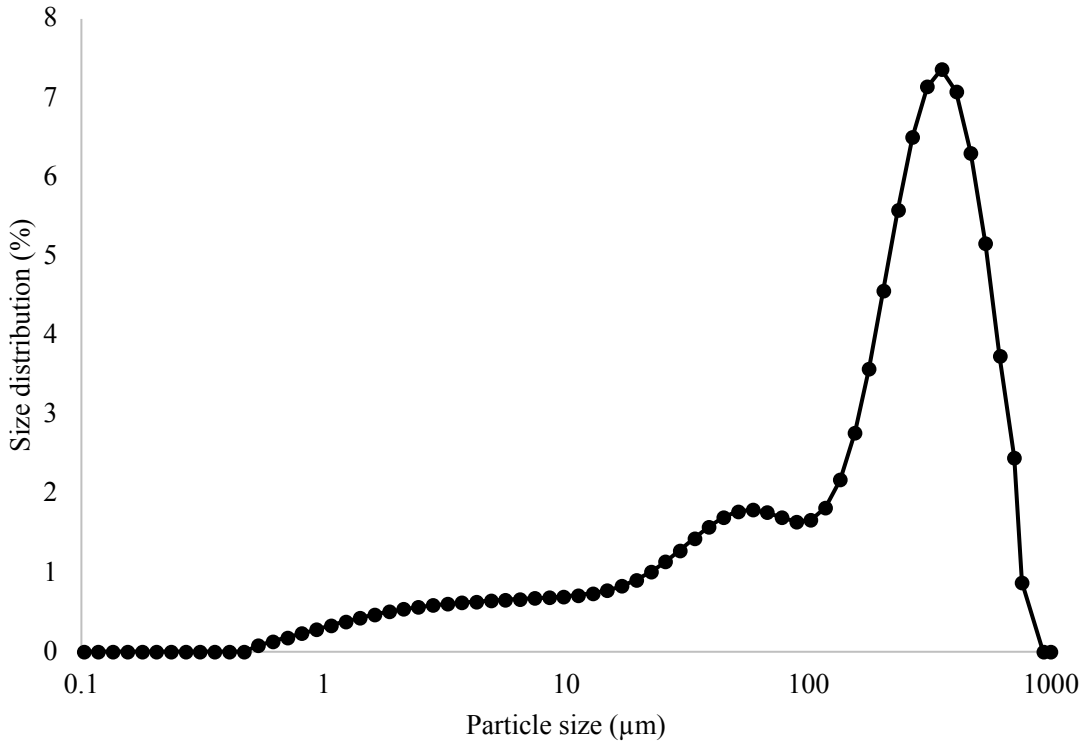
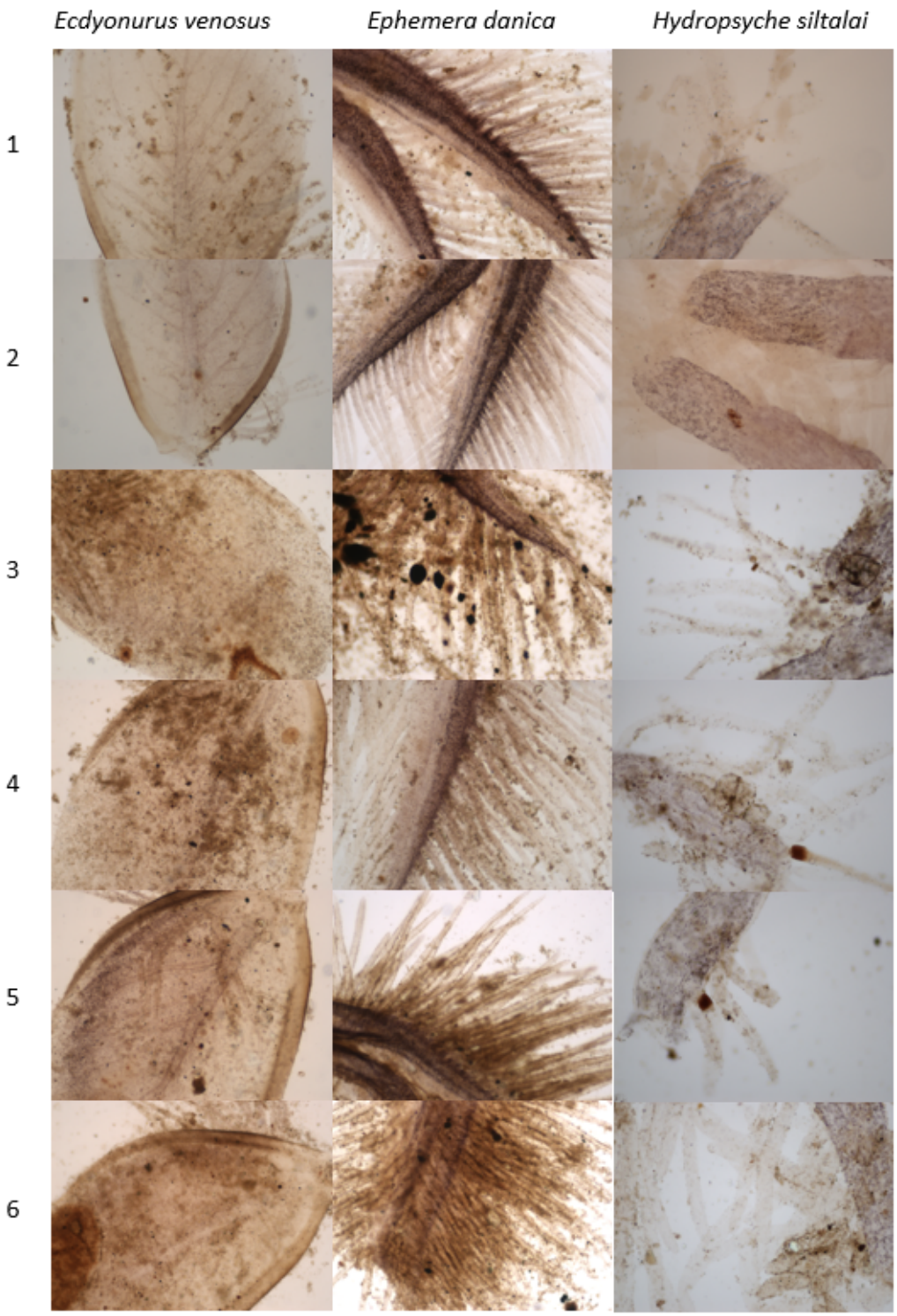


Figure S1. Particle size distribution curve of the sediment aggregate mix added to the recirculating flume system during the experiments. The particle size distribution was calculated using laser particle size analysis and is an average of two samples from each of two duplicate runs.



669

670 Figure S2. Images of slide mounts of invertebrate gills for each of *Ecdyonurus venosus* (10 X
671 magnification), *Ephemera danica* (10 X magnification) and *Hydropscyhe siltalai* (20 X
672 magnification) after exposure two controls and four treatments of varying SSC and flow velocity.
673 Control (1) = 3.5 mg l^{-1} at 0.19 m s^{-1}, control (2) = 3.5 mg l^{-1} at 0.37 m s^{-1}, treatment (3) = 83.7 mg l^{-1}
674 at 0.19 m s^{-1}, treatment (4) = 83.7 mg l^{-1} at 0.37 m s^{-1}, treatment (5) = 404.0 mg l^{-1} at 0.19 m s^{-1} and
675 treatment (6) = 404.0 mg l^{-1} at 0.37 m s^{-1}.

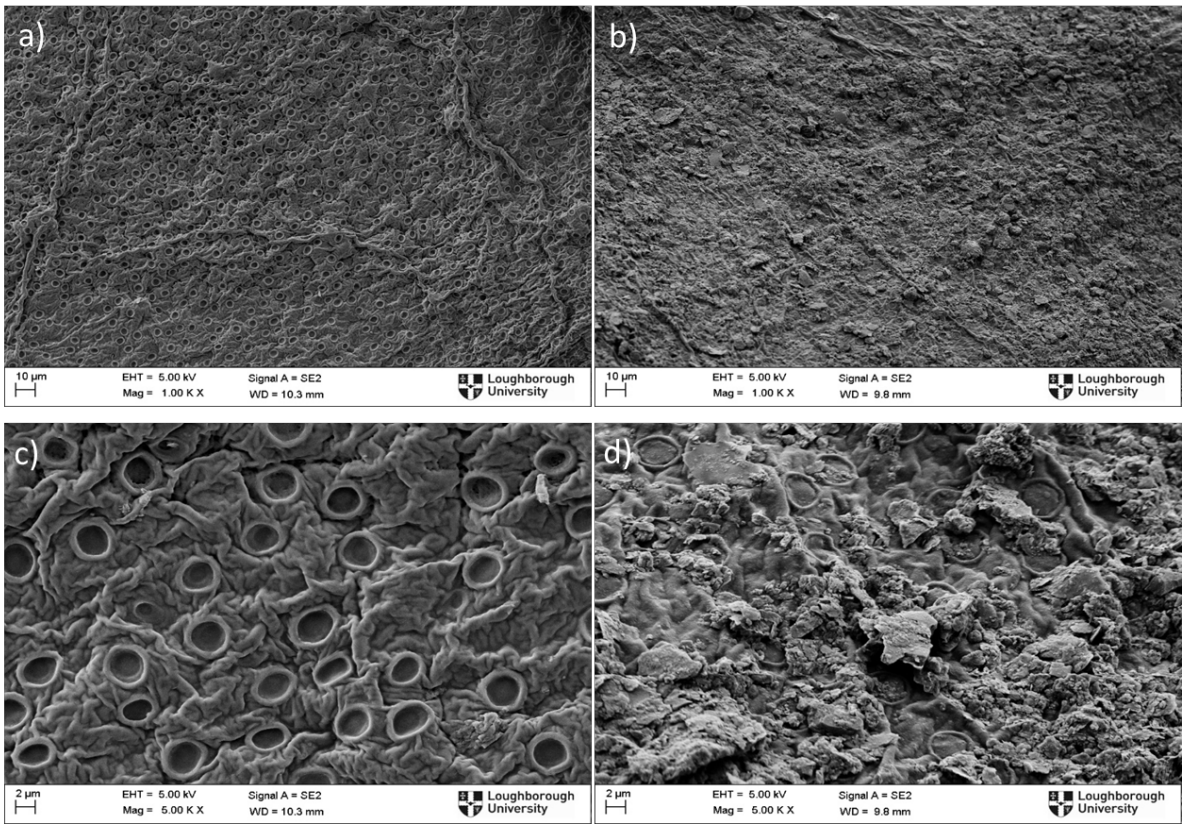


Figure S3. SEM images from *Ecdyonurus venosus* individuals which had been observed immediately after sampling (a and c) and those which had undergone sediment exposure as part of a pilot study (b and d).

Table S2. Summary results from the three-way ANOVA. *Denotes a significant term ($p<0.05$).

Term	**Df**	**SS**	**Estimate**	***F***	***p***
Species	2	1.41	0.70	29.50	2.23e-10*
Sediment	2	1.02	0.51	21.41	3.31e-08*
Velocity	1	0.05	0.05	1.96	0.16
Species:Sediment	4	0.83	0.21	8.67	6.92e-06*
Species:Velocity	2	0.27	0.14	5.67	4.94e-3*
Sediment:Velocity	2	0.05	0.02	0.96	0.39
Species:Sediment:Velocity	4	0.54	0.13	5.62	4.72e-04*
Residuals	82	1.95	0.24		

685 Table S3. Summary results from the model selection procedure. *Denotes that the model including
686 the interaction is a significantly better fit than the simpler model ($p<0.05$).

Model	**Res. Df**	**RSS**	**Df**	**SS**	***F***	***p***	**AIC**
Ecdyonurus venosus							
Sediment + Velocity	29	0.44					-38.61
Sediment * Velocity	27	0.40	2	0.05	1.53	0.23	258.78
Ephemera danica							
Sediment + Velocity	27	0.98					-9.20
Sediment * Velocity	25	0.88	2	0.09	1.37	0.27	268.95
Hhydropsyche siltalai							
Sediment + Velocity	32	1.12					-12.87
Sediment * Velocity	30	0.68	2	0.44	9.76	5.44e-04*	300.47

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